CONDUCTANCE OF IMPROPERLY FOLDED PROTEINS THROUGH THE SECRETORY PATHWAY AND RELATED METHODS FOR TREATING DISEASE

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CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of, and claims priority to, U.S.S.N. 09/427,696, filed October 27, 1999, which is herein incorporated by reference.

FIELD OF THE INVENTION

This invention provides the methodology and agents for treating any disease or clinical condition which is at least partly the result of endoplasmic reticulum-associated retention of proteins. Thus, the methods and agents of the present invention provide for the release of normally retained proteins from the endoplasmic reticulum. The present invention is particularly useful for treating any disease or clinical condition which is at least partly the result of endoplasmic reticulum-associated retention or degradation of mis-assembled or mis-folded proteins.

BACKGROUND

All publications and patent applications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

A. Introduction

Protein folding and quality control machinery has been implicated in the molecular pathogenesis of several human diseases caused by defective intracellular transport of an aberrantly folded protein through the secretory pathway. Exemplary diseases include pulmonary emphysema resulting from severe plasma α-antitrypsin deficiency and Cystic Fibrosis resulting from mutations in the cystic fibrosis transmembrane conductance regulator (Amara *et al.*, Trends Cell. Biol. 2:145-149; Le *et al.*, J. Biol. Chem. 269:7514-

7519; Pind *et al.*, J. Biol. Chem. 269:12784-12788). This invention is directed to the treatment and cure of such diseases.

Although the treatment and cure of Cystic Fibrosis and Chronic Obstructive Pulmonary Disease have been chosen as representative diseases for the purpose of describing and explaining the present invention, the compositions and/or methods of the present invention are applicable to the treatment and cure of any disease which involves the defective intracellular transport of mis-folded proteins.

B. Cystic Fibrosis - An Overview of the Disease, Protein and Gene

The Disease of Cystic Fibrosis. Cystic Fibrosis (CF) is an inherited multi-system metabolic disorder of the eccrine and exocrine gland function, usually developing during early childhood and affecting mainly the pancreas, respiratory system and sweat glands. Glands which are affected by CF produce abnormally viscous mucus, usually resulting in chronic respiratory infections, impaired pancreatic and digestive function, and abnormally concentrated sweat. CF is also called Clarke-Hadfield syndrome, fibrocystic disease of the pancreas and mucoviscidosis.

CF is the most common fatal autosomal recessive disease in Caucasians affecting approximately 1 in 2000 or 2500 live births, with 1 person in 25 being a heterozygote (Boat *et al.*, Metabolic Basis of Inherited Disease 2649-2680 (McGraw-Hill, 1989)). It is a complex disorder mainly affecting the ability of epithelial cells in the airways, sweat glands, pancreas and other organs and tissues to secrete chloride ions (Cl^-), leading to a severe reduction of the accompanying sodium and water in the mucus. Thus, the primary defect in CF is thought to be the relative impermeability of the epithelial cell to chloride ions (Cl^-). This defect results in the accumulation of excessively thick, dehydrated and tenacious mucus in the airways, with subsequent bacterial infections, mucus blockage and inflammation. For a detailed discussion of the clinical manifestations, diagnosis, complications and treatment of the disease, *see* R.C. Bone, Cystic Fibrosis, *In* J.C. Bennett et al., Cecil Textbook of Medicine 419-422 (W.B. Saunders Co., 1996).

The CF Protein and Gene. The gene for CF is located on the long arm of chromosome 7. For a description of the gene, the expression of the gene as a functional protein, and confirmation that mutations of the gene are responsible for CF, *see* Gregory *et al.*, Nature 347:382-386 (1990); Rich *et al.*, Nature 347:358-363 (1990); and Watson et al., Recombinant DNA, pp. 525-529 (Scientific American Books, 1992).

The protein encoded by the CF-associated gene is the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is a cyclic AMP-dependent chloride channel found

in the plasma membrane of certain epithelial cells. CFTR contains approximately 1480 amino acids and is made up of two repeated elements, each comprising six transmembrane segments and a nucleotide binding domain. The two repeats are separated by a large, polar, so-called R-domain containing multiple potential phosphorylation sites. Based on its predicted domain structure, CFTR is a member of a class of related proteins which includes the multi-drug resistance or P-glycoprotein, bovine adenyl cyclase, the yeast STE6 protein as well as several bacterial amino acid transport proteins (Riordan *et al.*, Science 245:1066-1073 (1989); Hyde *et al.*, Nature 346:362-365 (1990)). Proteins in this group are characteristically involved in pumping molecules into or out of cells.

Gene Mutations Responsible for CF. The metabolic basis for CF results from a mutational defect in a specific chloride channel. Naturally-occurring, single amino acid mutations have been found in the first nucleotide binding fold of CFTR. Although over 800 different mutations have been identified in the CF associated gene, the most common is a deletion of three nucleotides which results in the loss of a phenylalanine residue at position 508 of CFTR (ΔF508) (Davis *et al.*, Am. J. Respir. Crit. Care Med. 154:1229-1256 (1996); Sheppard and Welsh, Physiol. Rev. 79:Suppl: S23-S45 (1999)).

Additional examples of CFTR mutants include G551D, a mutation in the CFTR gene resulting in a substitution of aspartic acid for glycine at amino acid 551 of the CFTR (U.S. Patent No. 5,602,110), and several naturally-occurring CFTR mutants carrying a defect in the first nucleotide binding fold (NFB1) (U.S. Patent No. 5,434,086).

Mutations at position 508 contribute to approximately 90% of all CF cases, although the percentage varies by race and geographical location (Kerem *et al.*, Science 245:1073-1080 (1989)). This mutation results in the failure of an epithelial cell chloride channel to respond to cAMP (Frizzel *et al.*, Science 233:558-560 (1986); Welsh, Science 232:1648-1650 (1986); Li *et al.*, Science 244:1353-1356 (1989); Quinton, Clin. Chem. 35:726-730 (1989)). Although CF-affected epithelial cells are unable to normally up-regulate apical membrane Cl- secretion in response to agents which increase cAMP, they do increase Cl- secretion in response to increases in intracellular Ca^{2+}.

There are at least three different chloride channels found in epithelial cells, including volume sensitive, calcium-dependent and cAMP-dependent. In normal individuals, chloride channels are located on the luminal membranes of epithelial cells. When these channels are open, chloride ions move into the airway lumen, producing an osmotic gradient that draws water into the lumen. In Cystic Fibrosis, the absence or dysfunction of at least one of these chloride channels, CFTR, results in the failure to